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Short Communication

Isolation and characterization of microsatellite loci from the Chinese white wax scale *Ericerus pela* Chavannes (Homopetera: Coccidae)

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Eight microsatellite loci were isolated using an enrichment protocol from *Ericerus pela* Chavannes, representing the first microsatellite loci available for the white wax scale. All loci were polymorphic within *E. pela*. Allele numbers range from six to eleven with the observed heterozygosity (H_O) ranging from 0.0000 to 1.0000. These primers screened in this study provide a foundation for population genetic studies of the white wax scale.

Key words: Microsatellite marker, white wax scale, population genetic.

INTRODUCTION

The Chinese white wax scale is a famous resource insect of China, the wax secreted by the scale was widely used in wax candle production, wax printing, engraving print and Chinese medicine in China about one thousand years ago, while it is now been used in chemical, pharmaceutical, food and cosmetic industry (Chen and Feng, 2009). Because of wax large application, the study of the scale secreted wax become more and more important. The white wax scale widely distributed in most parts of China, Japanese and Korea peninsula from subtropics region (north latitude 24°) to temperate region (north latitude 44°) and formed different geographic populations adapting to different climate (Long, 2004; Wang and Xu, 2006; Chen and Feng, 2009). Such adaptation has a strong impact on the genetic variation among populations (Chen et al., 1998). For population genetic analyses and breeding studies of the white wax scale, it is important to isolate and characterize its microsatellite loci.

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MATERIALS AND METHODS

Microsatellite-containing DNA fragments library construction

Genomic DNA was isolated from a single *Ericerus pela* female adult individual following the 'proteinase K' protocol described by Tian (1999). DNA was digested with *Rsa*I restriction enzyme (TaKaRa) then ligated to 21 linker (5'-CTCTTGCTTACGCGTGGACTA-3') and 25 linker (5'-phosphate-TAGTCCACGCGTAAGCAAGAG-3'). The enrichment procedure was followed by the protocol from Glenn and Schable (2005) and Franck et al. (2005) based on Streptavidin MagneSphere (Ivitrogen) with slight modifications. 5'-biotinylated $(CT)_{10}$ and $(GT)_{10}$ oligonucleotides were used as probes. The enriched single strand DNA was amplified by PCR (polymerase chain reaction) using *Rsa*21 linker as primer to recover double-stranded DNA. The 250 to 1000 bp PCR fractions were selected on an agarose gel, purified, and ligated into pMD18 - T vector (TaKaRa). The plasmids were further transformed into *Escherichia coli* competent cells (Tiangen).

Microsatellite loci sequencing, primer design and evaluation

About one hundred recombinant clones propagated in LB/ampicillin medium were selected at random and sequenced by Huada Genetics Company. A total of 31 sequences contained clear repeated units (di, tri and tetranucleotide). Following primers were

Table 1. Characterization of eight microsatellite loci cloned from *Ericerus pela* Chavannes, described by locus name, forward (F) and reverse (R) primer sequences, repeat motif, optimal annealing temperatures (T_a), size of allele (Size), size range of alleles, number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E) and significance of departure from Hardy–Weinberg equilibrium (HWE). Polymorphism statistics were performed on 24 individuals.

Locus	Repeat	Primer sequences (5'-3')	GenBank accession no.	Size (bp)	Size range (bp)	Ta (°C)	N_A	H_O	H_E	HWE *p* value
Pela1	$(GTGAT)_6(GT)_8(GCGT)_5$	F: ACTAACGCAAGCGCAGCTAT R: TGCAGCTAGTCGCCACAATA	HQ384427	196	160-232	58	8	0.7500	0.8511	0.241
Pela2	$(AC)_{12}$	F: TTTGTAGTGAAACTTCTTTAG R: GCTCTTATGTTGTTGTATG	HQ641376	153	147-175	55	6	0.0000	0.7872	0.000*
Pela6	$(TTTG)_3TTTA(TTTG)_4$	F: GGACTAACGAACCGAGTCCA R: AAAGCACAAAAATGAAGTGAACA	HQ384423	236	196-256	58	6	0.0000	0.7837	0.000*
Pela17	$(AC)_{18}$	F: AGCACAACACACCACAGCTC R: GACTACCATGGGCACGAACT	HQ384426	182	196-228	58	10	1.0000	0.8972	0.000*
Pela60	$(TG)_{14}$	F: AGGTCTGCAGTTGTCCCATT R: AATATTGCTGCGCTGGAGTT	HQ384428	194	174-236	50	6	0.0000	0.8710	0.000*
Pela63	$(AC)_8$	F: TGTTCATTGGCGAAGCTTTA R: TGTTTTTGCATCCACACGTT	HQ384430	175	171-199	50	11	0.9167	0.9069	0.048*
Pela70	$(AC)_3(AT)_2(AC)_8$	F: CGTTTAGAACAACGCAGCAG R: TCGCTTAGTATTTGCACACAA	HQ384424	184	172-234	58	11	1.0000	0.9087	0.004*
Pela81	$(AC)_8(ACGC)_6$	F: ATACACAAGCGCACGCACA R: CGCGTGGACTAACACATATAGC	HQ384425	108	98-112	58	6	0.0000	0.7949	0.000*

*Significant departure from HWE.

designed for these sequences using Primer 3 online.

To evaluate the polymorphism of the primer pairs designed, 24 *E. pela* individuals collected from *Ligustrum lucidum* trees in a greenhouse in Kunming were used. PCR reactions were carried out in 10 l reaction volumes containing 1×PCR buffer, 2 m $MMgCl_2$, 0.2 mM dNTP, 1 U *Taq* DNA polymerase (TaKaRa), 0.5 M each primer and 0.5 l DNA template. For all tested primers, thermal cycling conditions were carried out as follows: 4 min at 94°C, 30 cycles were performed consisting of 45 s at 94°C, 45 s annealing (Table 1 shows annealing temperatures) and 30 s extension at 72°C, with a final extension step at 72°C for 10 min. PCR productions mix with GelRed (Biotium, America) were separated on 8% denaturing polyacrylamide gels.

Observed heterozygosity (H_O), expected heterozygosisty (H_E) and significant deviations from Hardy-Weinberg equilibrium (HWE) were calculated using POPgene 1.32 (Yeh et al., 2000). Linkage disequilibrium (LD) and frequency of null allele were tested using GENEpop 4.0 on the web.

RESULTS AND DISCUSSION

Among the primer pairs tested in this laboratory

population, eight were polymorphic and two were monomorphic. Summary data for the eight characterized polymorphic loci are presented in Table 1. These data revealed that observed heterozygosity of all loci (HO) varied between 0.0000 and 1.0000, HO of four homozygous loci were 0.0000, and two heterzygous loci were 1.0000. These homozygous/heterzygous loci departure from Hardy-Weinberg equilibrium significantly could be due to sampling bias (*E. pela* individuals collected from only one isolated greenhouse). Perhaps no genetic linkage between these loci, homozygote and heterozygote were kept in the greenhouse) and unknown founder relatedness in the semi-free population. We compared the results of *E. pela* to other species (Vuuren, 2006; Miao et al., 2008; Lavandero and Dominguez, 2010; Zou et al., 2010), such as *Platyscapa awekei* (common deviation from HWE due to inbreeding), *Eriosoma lanigerum* (small proportion of loci deviating from HWE due to null alleles), *Grus japonensis* (sampling bias and unknown founder relatedness) and *Taxus yunnanensis* (most loci significantly deviated from HWE due to a large heterozygote deficiency). Expected heterozygosity (HE) of the eight loci range from 0.7872 to 0.9087. No significant LD was detected among the eight loci. These primers were used in other geographic populations of *E. pela* successfully.

In conclusion, we report the isolation of eight microsatellite DNA loci which are useful for population genetic analyses of the *E. pela*. Further studies using these primers should allow a better understanding of phylogeographic relationships and the origin land of the white wax scale.

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